CLAIMS

I claim:

1. An improved apparatus for identification of biomolecules comprising:

   a planar semiconductor substrate having a top side, a bottom side, and external electrical contacts;

   a plurality of sense sites formed within the substrate top side further defining a matrix of sense sites, wherein each sense site comprises a four sided well constructed from silicon dioxide isolation layers, a gap to receive probe molecules, a gap substrate capable of changing its resistivity with appropriate processing serving as the bottom of the four sided well, means to affix probe molecules in the sense site gap to the gap substrate, means to electrically detect biomolecules within the gap, means to vary base resistivity across the sense site gap, and means to break surface tension of sample liquids;

   means to control the semiconductor substrate temperature;

   means to protect the semiconductor substrate top side;

   means to sequentially read parallel electrical measurements from multiple sense sites and correlate output data between all sense sites allowing competitive hybridization, chip-to-standard, or chip-to-chip baseline measurements;

means to separate a spot sample applied to gaps in the matrix from nearby sense sites not in contact with the sample; and

means to separate a spot sample into multiple, separate sense site wells.

2. The apparatus of claim 1, wherein the substrate is silicon.

3. The apparatus of claim 1, wherein the substrate is germanium.

4. The apparatus of claim 1, wherein means to affix probe molecules in the sense site gap to the gap substrate further comprises a bioreactive material selected from carbon, hydrophillic organic polymers, inorganic metal oxides and inorganic metal nitrides.

5. The apparatus of claim 1, wherein the substrate further comprises a diode or unidirectional semiconductor device.

6. The apparatus of claim 1, wherein means to electrically detect biomolecules within the gap further comprises two conductive traces located on opposite sides of the sense site gap, wherein the variable resistance gap substrate separates the conductive traces and is connected to the conductive traces, a unidirectional electrical element in series with one of the two conductive traces, wherein the plurality of sense sites forms a two dimensional matrix on the substrate top side which, except for the sense site addressed, energizes the sense site electrical elements in a reverse biased mode, and wherein each sense site is

addressable and reliably read using microprocessors, microcontrollers, multiplexers, demultiplexers, addressable switches, temperature sensitive elements, and amplifier circuits produced on the substrate.

7. The apparatus of claim 1, wherein means to affix probe molecules in the sense site gap to the gap substrate further comprises a functionalizing reagent applied to the gap substrate.

8. The apparatus of claim 1, wherein means to protect the substrate top side further comprises a passivation layer coating disposed on the substrate top side.

9. The apparatus of claim 1, wherein means to separate a spot sample applied to gaps in the matrix from sense sites not in contact with the sample and means to separate a spot sample into multiple, separate sense site wells further comprise at least one metallic or magnetic bead of sufficient diameter moving on the substrate top side so that the bead cannot enter or drop down into the sense site well, and wherein means for movement of the bead on the substrate top side consists of substrate motion, external permanent magnets or electromagnets, or a varying external magnetic field.

10. The apparatus of claim 1, wherein means to break surface tension of sample liquids further comprises at least one liquid shunt.

11. The apparatus of claim 1, wherein means to control substrate temperature further comprises a temperature sensitive diode connected to the external contacts of the substrate.

12. The apparatus of claim 6, wherein the conductive traces further comprise rounded edges.

13. The apparatus of claim 6, wherein the gap substrate further comprises non-oxidized semiconductor material.

14. The apparatus of claim 1, wherein means to sequentially read parallel electrical measurements from multiple sense sites and correlate output data between all sense sites allowing competitive hybridization, chip-to-standard, or chip-to-chip baseline measurements comprises a portable or plug-in test instrument.

15. The apparatus of claim 14, wherein means to sequentially read parallel electrical measurements from multiple sense sites and correlate output data between all sense sites allowing competitive hybridization, chip-to-standard, or chip-to-chip baseline measurements further comprises a portable analysis instrument comprising a chamber to perform liquid processing of the array/sense site and means to read, store, and transmit measurements of sense site resistance or conductance.

16. The apparatus of claim 15, wherein means to sequentially read parallel electrical measurements from multiple sense sites and correlate output data between all sense sites allowing competitive hybridization, chip-to-standard, or

chip-to-chip baseline measurements further comprises a fast comparator circuit that will signal when potentially damaging high currents are present on the chip and disconnect and protect the sense chip from the fault.

17. The apparatus of claim 1, wherein spacing of sense sites is not more than 50 microns from the center of a sense site gap to the center of the next closest sense site gap in any direction.

18. The apparatus of claim 1, wherein at least three neighboring sense sites each contain sample aliquots from the same, single, mechanical application or synthetic construction of a probe spot in the region, and wherein each sense site analyses its respective aliquot independently of all other sense sites.

19. The apparatus of claim 1, wherein the substrate further comprises a programmable fuse link circuit which allows the mean circuit resistance to be adjusted to specific data sheet values.

20. The apparatus of claim 1, wherein the substrate further comprises a temperature sensitive element such as a diode which produces a signal characterized to determine and control substrate temperature.

21. The apparatus of claim 1, wherein the substrate further comprises a matrix of closed, conductive sense sites for parametric testing and wherein which, during manufacture, the closed sense sites are opened using laser trimming

techniques to preserve the substrate's unidirectional diode matrix after testing.

22. A method for identification of biomolecules employing probe spot application to a semiconductor sense chip substrate having a top side and comprising a plurality of sense sites formed within the substrate top side as an array surface with each sense site further comprising an amino-silane or metal oxide sense site well having a predetermined and uniform width, length, and depth dimension and a sense site substrate, the method comprising the steps of:

a. denaturing probe spot DNA by heating to 95 degrees C for 15 minutes;

b. applying all probe spots to the array surface and drying;

c. coating the array surface with salmon sperm solution;

d. allowing the coated array surface to stand for a predetermined period of time;

e. rinsing the coated array surface with 2X SSC;

f. preparing a solution of 2X SSC and latex coated beads with a diameter at least 10X larger than the sense site well length;

g. placing the substrate on a magnetic base instrument;

h. coating the substrate top surface with the bead solution;

i. switching on the magnetic base instrument;

j. allowing the substrate to be treated by the magnetic base instrument for a predetermined period of time;

k. immediately rinsing the substrate top surface with 2X SSC twice at room temperature for a predetermined period of time for each wash;

l. allowing the substrate top side to dry while being stored in a covered environment;

m. applying means to establish covalent bonds to the sense site substrate;

n. taking baseline electrical measurements; and

o. covering and storing the substrate until ready for hybridization.

23. The method of claim 22, wherein the means to establish covalent bonds to the sense site substrate further comprises the step of baking the substrate at 80 degrees centigrade for 80 minutes.

24. The method of claim 22, wherein the means to establish covalent bonds to the sense site substrate further comprises the step of applying UV energy using a UV crosslinker.

25. A method for identification of biomolecules employing probe spot application to a semiconductor sense chip substrate having a top side and comprising a plurality of sense sites formed within the substrate top side as an array surface with each sense site further comprising an amino-silane or metal oxide sense site well having a predetermined and

uniform width, length, and depth dimension and a sense site substrate, the method comprising the steps of:

a. preparing a stationary probe spotting solution;
b. spotting the stationary probe to the sense chip;
c. fixing stationary DNA to and blocking the sense chip;
d. preparing biotin labeled target DNA;
e. preparing a hybridization mixture;
f. hybridization of target to the sense chip;
g. washing the sense chip;
h. applying streptavidin-gold conjugate;
i. enhancing the streptavidin-gold conjugate with silver; and
j. measuring the sense chip.

26. The method of claim 25, wherein the steps of preparing a stationary probe spotting solution and spotting the stationary probe to the sense chip further comprise the sub-steps of:

a. placing 2μg of probe DNA in at least 10μL of a solution containing 10% DMSO and dH_2O for each probe spot;
b. heating the DNA mixture to 95 degrees centigrade for 15 minutes and then placing the mixture on ice;
c. positioning the probe DNA samples in an appropriate container (864 well plate) and setting the container into a probe spotting machine;

d. operating machine and spotting each probe onto a separate sense chip; and

e. allowing each spotted sense chip to air dry and storing the dried, spotted sense chips in a covered environment at room temperature.

27. The method of claim 25, wherein the step of blocking and fixing stationary DNA to the sense chip further comprises the sub-steps of:

a. to a clean 1.5 mL tube, adding 25 µL of Master mix (0.1g dextran sulfate, 5mL formamide, and 1 mL 20X SSC and water up to 7 mL, pH 7.0) and enough fractionated Salmon Sperm DNA to reach a concentration of 250 µg/mL;

b. heating the mixture to 37 degrees centigrade and quickly applying it to the surface of the sense chip, covering the sense chip cavity with a supplied plastic cover, and placing the sense chip on a slow rocker platform in 37 degrees centigrade incubator for 30 minutes;

c. rinsing the sense chip twice with 2X SSC solution at 45 degrees centigrade for 5 minutes;

d. in a separate test tube, combining 2 µL of magnetic beads with 23 µL of 2X SSC and applying the combination to the well of the sense chip;

e. securing the sense chip on a magnetic stirrer base and covering the sense chip with the plastic cover provided while the magnetic stirring function is applied to the sense chip for 5 minutes;

f. removing the sense chip from the platform and quickly rinsing the sense chip in 2X SSC at 45 degrees centigrade for 5 minutes;

g. rinsing the sense chip a final time for 5 minutes in 0.1X SSC at 45 degrees centigrade;

h. allowing the sense chip to air dry for 10 minutes and, if desired, replacing plastic cover onto the sense chip; and

i. placing the sense chip into a testing machine and reading the resistance and/or conductance levels of each sense site.

28. The method of claim 25, wherein the step of preparing Biotin labeled target DNA further comprises the sub-steps of:

a. taking sample DNA and preparing a random primer PCR mix with biotin labeled dUTPs, following normal PCR procedures wherein the random primer PCR mix comprises 25 µL PCR reaction and includes dUTP-biotin: dTTP in ratio of 1: 3 in the PCR mix, which follows the sub-steps of (i) mixing 0.2 µg of sample DNA (approx. 1 µL), 10 µL of 2.5X Random Primer, and 9.8 µL water, (ii) denaturing the mixture in a PCR machine at 100 degrees centigrade for 10 minutes, (iii) adding 2.5uL dNTPs, 1.0 µL Biotin labeled dUTP, and 0.7 µL Klenow Fragment for an approximate total volume of 25 µL, and

(iv) mixing well and incubating for 4 hours at 37 degrees centigrade;

b. upon completion, running a 1% Agarose gel and DNA ladder to determine if the biotin labeled target sequences have been evenly generated wherein the biotin sample should show a smear with the darkest areas between 200bp and 800bp;

c. using an Amersham MicroSpin G-50 column, snapping open the columns and placing in a new 1.5 mL tube;

d. spining at 770 rcf for 1 minute to remove excess buffer;

e. discarding the buffer and replacing the column into the 1.5mL tube;

f. applying the biotin labeled target solution and spinning again at 770 rcf for 2 minutes; and

g. discarding the column and placing the tube with target DNA on ice.

29. The method of claim 25, wherein the step of preparing a hybridization mixture comprises the sub-steps of:

a. combining 25 µL of the Target DNA mixture with 25 µL (2 µg)of Cot 1 DNA in a 1.5 mL tube and ethanol precipitate, which further comprises 2.5 volumes of ice cold ethanol (100%) and 0.1 volume of 3M sodium acetate pH 5.2, with 25 µL of Target DNA;

b. spining the combination at 14,000 rpm for 30 minutes at 4 degrees centigrade;

c. decanting the supernatant and air drying the pellet for 10 minutes, using a rolled Kimwipe to remove any excess Ethanol;

d. resuspending the Target pellet in 5 µL water, 10 µL 20% SDS, and 35 µL of Mastermix, which further comprises 0.1 gram dextran sulfate (mw approximately 500,000), 5 mL of formamide, 1 mL 20X SSC, and water up to 7 mL at pH 7.0;

e. after the pellet is resupended, denaturing the Target mixture at 75 degrees centigrade for 15 minutes; and

f. removing the Target mixture to a 37 degrees centigrade incubator and allowing the Cot 1 DNA to preanneal to the Target for a minimum of 1 hour, thus blocking repetitive sequences in the target.

30. The method of claim 25, wherein the step of hybridization of target to the sense chip comprises the sub-steps of:

a. rinsing the sense chip in 2X SSC for 5 minutes at 45 degrees centigrade and air drying the rinsed sense chip for 5 minutes;

b. placing the sense chip into a Stratalinker UV machine and applying 2600 x 100 µjoules to covalently link the Probe DNA to the surface of the sense gaps;

c. applying the warm target mixture to the sense chip surface;

d. sealing the sense chip into a plastic hybridization chamber provided;

e. placing the assembly on a slow rocker platform in an incubation oven at the researcher's prescribed temperature and for a predetermine period of time.

31. The method of claim 25, wherein the step of washing the sense chip comprises the sub-steps of:

a. pre-warming the wash solutions of wash 1 (50% formamide and 50% 1X SSC), wash 2 (2X SSC), wash 3 (0.1X SSC), and wash 4 (PN Buffer 0.1M sodium phosphate with 0.1% NP-40) to 45 degrees centigrade in large Coplin jars;

b. Rinsing/soaking the array sense chip in above rinse solutions for the following time periods for each solution (i) Formamide for 10 minutes, (ii) 2X SSC for 10 minutes, (iii) 0.1X SSC for 5 minutes, and (iv) PN Buffer for 5 minutes;

c. shaking the last remaining liquid from the sense chip well and allowing the sense chip to air dry for 15 minutes.

32. The method of claim 25, wherein the step of applying streptavidin-gold conjugate comprises the sub-steps of:

a. preparing a 25 µL streptavidin/gold solution in amber tubes comprising a 1:4 dilution of streptavidin gold into 15mM NaCl solution and 0.1% BSA;

b. pipetting the streptavidin/gold solution onto the sense chip and covering the sense chip with the plastic sense chip cover;

c. placing the sense chip on a slow rocker in an incubator at 37 degrees centigrade for 30 minutes;

d. rinsing the sense chip with 1 X SSC for 1 minute;

e. rinsing the sense chip with 0.1X SSC for 1 minute; and

f. allowing the sense chip to air dry for 15 minutes.

33. The method of claim 25, wherein the step of enhancing the streptavidin-gold conjugate with silver comprises the sub-steps of:

a. rinsing the sense chip in double distilled water at 45 degrees centigrade for 1.5 minutes to remove sodium ions;

b. preparing a 100 µL solution of 2M sodium citrate, 0.5M hydroquinone, and 0.03M silver lactate solution in a dark room, and pouring 30 µL of the prepared solution onto the sense chip and letting the treated sense chip incubate at room temperature for 3 minutes;

c. rinsing the sense chip with 1% acetic acid solution and allowing the treated sense chip to incubate in 30 µL of this solution for 2 minutes;

d. rinsing the sense chip with Kodak® Rapid Fix® fixative solution and allowing the treated sense chip to incubate in 30 µL of this solution for 3 minutes at room temperature;

e. rinsing the sense chip in double distilled water for 1.5 minutes at room temperature, then rinsing the sense chip for 3 minutes in 0.1% SSC at room temperature for 3 minutes;

f. washing the sense chip in double distilled water for 30 seconds at room temperature wash to complete the silver enhancement, and

g. allowing the silver enhanced sense chip to dry.

34. The method of claim 25, wherein the step of measuring the sense chip shows probe/target hybridization to be successful when deceased resistivity of the sense sites after enhancing the streptavidin-gold conjugate with silver compared with the original resistance readings made prior to hybridization of the target.

35. The apparatus of claim 1, wherein the semiconductor substrate comprises various resistive sense sites.

36. The apparatus of claim 1, wherein a plurality of semiconductor substrates comprise a substrate family of various resistive sense sites.